Cyclophilin A complexed with a fragment of HIV-1 gag protein: insights into HIV-1 infectious activity

Yingdong Zhao[1], Yongquan Chen[1], Mike Schutkowski[2], Gunter Fischer[2] and Hengming Ke[1]*

Background: Cyclophilin A (CyPA), a receptor of the immunosuppressive drug cyclosporin A, catalyzes the *cis-trans* isomerization of peptidyl–prolyl bonds and is required for the infectious activity of human immunodeficiency virus type 1 (HIV-1). The crystal structure of CyPA complexed with a fragment of the HIV-1 gag protein should provide insights into the nature of CyPA–gag interactions and may suggest a role for CyPA in HIV-1 infectious activity.

Results: The crystal structure of CyPA complexed with a 25 amino acid peptide of HIV-1 gag capsid protein (25-mer) was determined and refined to an R factor of 0.195 at 1.8 Å resolution. The sequence Ala88-Gly89-Pro90-Ile91 of the gag fragment is the major portion to bind to the active site of CyPA. Two residues of the 25-mer (Pro90-Ile91) bind to CyPA in a similar manner to two residues (Pro-Phe) of the CyPA substrate, succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (AAPF). However, the N-terminus of the 25-mer (Ala88-Gly89) exhibits a different hydrogen-bonding pattern and molecular conformation than AAPF. The peptidyl–prolyl bond between Gly89 and Pro90 of the 25-mer has a *trans* conformation, in contrast to the *cis* conformation observed in other known CyPA–peptide complexes. The residue preceding proline, Gly89, has an unfavorable backbone conformation usually only adopted by glycine.

Conclusions: The unfavorable backbone conformation of Gly89 of the gag 25-mer fragment suggests that binding between HIV-1 gag protein and CyPA requires a special sequence, Gly-Pro. Thus, in HIV-1 infectivity, CyPA is likely to function as a chaperone, rather than as a *cis-trans* isomerase. However, the observation of similarities between the C termini of the 25-mer and the substrate AAPF means that the involvement of the *cis-trans* isomerase activity of CyPA cannot be completely ruled out.

Addresses: [1]Department of Biochemistry and Biophysics, School of Medicine, The University of North Carolina, Chapel Hill, NC 27599, USA and [2]Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V., Arbeitsgruppe 'Enzymologie der Peptidbindung', Kurt-Mothes-Straße 3, D-06120 Halle/Saale, Germany.

*Corresponding author.
E-mail: hke@med.unc.edu

Key words: chaperone, *cis-trans* isomerization, immunophilin, X-ray crystallography

Received: 28 October 1996
Revisions requested: 22 November 1996
Revisions received: 11 December 1996
Accepted: 11 December 1996

Electronic identifier: 0969-2126-005-00139

Structure 15 January 1997, 5:139–146

Introduction

Cyclophilin (CyP) binds the immunosuppressive drug cyclosporin A (CsA) [1], and is also an enzyme with peptidyl–prolyl *cis-trans* isomerase activity [2,3]. The CyP–CsA complex binds to calcineurin, a serine/threonine phosphatase and a Ca^{2+}-dependent calmodulin-binding protein [4–6].

The isomerization of peptidyl–prolyl amide bonds forms a slow step in protein folding, and CyP was reported to accelerate protein or oligopeptide folding [7–16]. The cyclophilin homolog NinaA was shown to form a stable complex *in vivo* with rhodopsin, and to function as a chaperone [17]. In addition, a chaperone complex, required for the folding of steroid receptors, was formed between cyclophilin-40, heat shock proteins (hsp70 and hsp90), and the protein p23 [18].

Cyclophilin A (CyPA) has been reported to bind human immunodeficiency virus type 1 (HIV-1) gag protein [19], and is required for the infectious activity of HIV-1 virions [20–22]. HIV gag is a polyprotein and is cleaved by proteases into several functional proteins: matrix (MA), capsid (CA), nucleocapsid (NC), and small peptides [23,24]. HIV-1 CA protein (also known as gag p24 or p24) is essential for the assembly and infectivity of HIV virions [23]. Binding of CyPA to the HIV-1 gag protein is mediated by the central region of CA [25–27]. Recently, the NMR structure of the N-terminal domain of CA (residues 1–151) [28] and the partial crystal structure of CA complexed with an Fab fragment [29] have been determined.

Three-dimensional structures have been reported for the unligated recombinant human T cell CyPA [30,31] and CyPA complexed with various proline-containing peptides: *N*-acetyl-Ala-Ala-Pro-Ala-amido-methylcoumarin (AAPA) [32,33], Ala-Pro [34], Ser-Pro, His-Pro, Gly-Pro [35], succinyl-Ala-Pro-Ala-*p*-nitroanilide (APA) [36], and succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (AAPF) [37]. These structures have provided insight into the mechanism of

the peptidyl–prolyl *cis-trans* isomerization. We report here the structure of CyPA complexed with a 25 amino acid peptide (25-mer) from the HIV-1 gag CA protein (residues 81–105 of gag). This structure indicates the probable nature of the interactions of CyPA with the HIV-1 gag protein, and suggests a role for CyPA in HIV-1 infectious activity.

Results and discussion

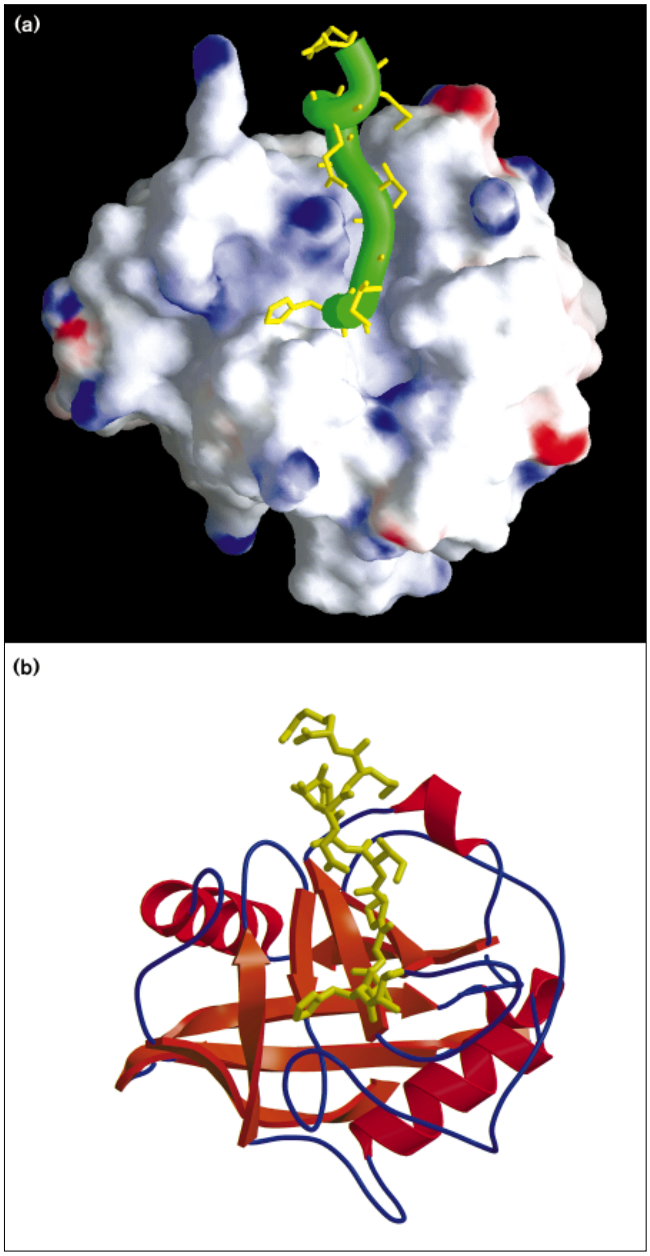
Binding of the 25-mer does not change the CyPA structure

The structure of CyPA complexed with the 25-mer of gag CA is an eight-stranded β barrel (Fig. 1), similar to the structures of unligated CyPA [30,31] and its complexes with proline-containing peptides [32–37]. The superposition of the CyPA–25-mer structure onto unligated CyPA, or its complex with the tetrapeptide AAPF, revealed an average positional shift of 0.27 Å for the Cα atoms. This observation indicates that binding of the 25-mer does not induce substantial changes on the structure of CyPA. However, in unligated CyPA a loop of six residues (Ala101–Asn106) moved significantly to accommodate the binding of the 25-mer fragment, as shown by its Cα positional shift of 0.86 Å; this shift is more than three times the overall average Cα shift of 0.27 Å. A similar movement was also observed in the structure of CyPA–AAPF; the average Cα shift is 0.97 Å for Gly104–Pro105 and 0.58 Å for Ala101–Asn106, relative to unligated CyPA.

The electron density was excellent for most residues of CyPA. The N-terminal methionine, which was not visible in the crystal structures of the unligated or peptide-complexed CyPA [31,34,35,37] was well ordered in the CyPA–25-mer structure. The average B factor was 30.6 $Å^2$ for the N-terminal Met1, and is comparable with the overall average B factor of 24.8 $Å^2$ for all atoms of CyPA.

The 25-mer gag fragment was partially observable: residues Val86–Arg97 (gag numbering) were well ordered (Fig. 2) while the N terminus (Asp81–Pro85) and the C terminus (Glu98–Ala105) were not traceable because of lack of electron density. However, when we looked carefully at the crystallographic lattice packing, there was some extra space available that might be occupied by disordered regions of the ligand. The 25-mer has been reported to bind CyPA in solution by circular dichroism (CD) spectroscopy and has further been characterized by two-dimensional exchange ^{1}H-NMR spectroscopy as a substrate for CyPA (U Reimer *et al.*, unpublished data). Thus, it seems likely that the 25-mer binds to CyPA in the similar buffer system used for crystallization, and that the complete 25-mer is present in the crystal with disordered N and C termini. We cannot exclude the possibility of some unknown cleavage. The average B factor was 36.4 $Å^2$ for all the atoms of Val86 to Arg97 of the 25-mer. This B factor is slightly higher than the average B factor of 24.8 $Å^2$ for CyPA. However, the average B factor for Ala88–Gly94 of the 25-mer, which forms the central portion for binding, was 29.1 $Å^2$ implying ordered binding in a 1:1 ratio of CyPA:25-mer. The high B factor for the N and C termini may reflect their thermal mobility and lack

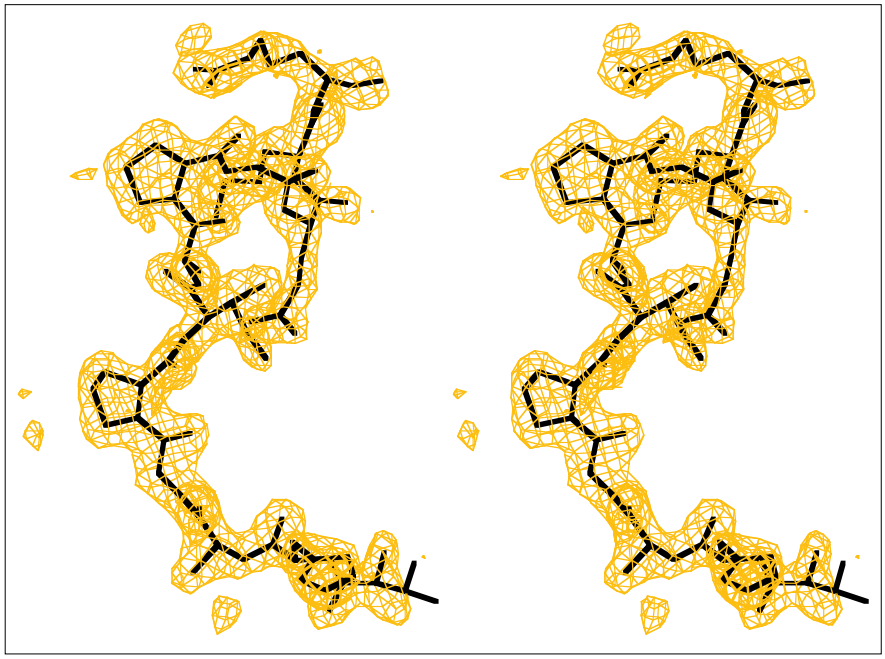
Figure 1



Crystal structure of CyPA complexed with a fragment of HIV-1 gag protein. (a) Molecular surface charge distribution: the positively charged regions are shown in blue and negatively charged regions are in red. The 25-mer of gag capsid protein is represented in green and its sidechains are in yellow. (Figure drawn using GRASP [49].) (b) Ribbon representation: helices are shown in red, β strands in orange and loops in blue. Residues of the 25-mer are shown as a yellow stick model. (Figure plotted using the program MOLSCRIPT [50] and Raster3D [51].)

Figure 2

Stereo plot of electron density of the 25-mer fragment. Residues of Asp81–Pro85 and Glu98–Ala105 were not visible in the electron-density maps. The residues shown in thick lines are: Val86-His87-Ala88-Gly89-Pro90-Ile91-Ala92-Pro93-Gly94-Gln95-Nle96-Arg97. The electron density shown as thin lines is calculated from the refined structure in which the 25-mer fragment was omitted. The ($2F_o$–F_c) map is contoured at 1.0σ. The peptide bonds of all the proline residues in the bound 25-mer fragment have a *trans* conformation.



of interaction with CyPA. The peptide conformational angles of ϕ and ψ for all residues of CyPA, and for the traced 25-mer fragment with the exception of glycine, were located within the energy-allowed regions of a Ramachandran plot [38].

Interactions between the 25-mer and CyPA

The 25-mer fragment of HIV-1 gag was bound at the active site on the molecular surface of CyPA. Fourteen CyPA residues (Arg55, Ile57, Phe60, Met61, Gln63, Asn71, Gly72, Thr73, Ala101, Asn102, Gln111, Phe113, Leu122 and His126) were involved in interactions with nine 25-mer residues (Val86, His87, Ala88, Gly89, Pro90, Ile91, Ala92, Pro93 and Nle96) (Table 1). The 25-mer has the same N- to C-terminal peptide chain direction as the tetrapeptide AAPF, but is in the opposite direction to that of CsA [39–42]. The sidechain of Pro90 of the 25-mer occupies the hydrophobic pocket of CyPA, which is made up of residues of Phe60, Met61, Phe113, Leu122 and His126. There are eight hydrogen bonds between the 25-mer and CyPA (Table 1; Fig. 3). Six of these hydrogen bonds involve the backbone of Ala88–Ile91 of the 25-mer, while two are formed between the sidechains of His87 and Gln95 of the 25-mer and the Asn71 carbonyl oxygen and the Arg55 sidechain of CyPA. In addition, six water molecules form hydrogen bonds with the 25-mer fragment (Table 1). These findings are consistent with the results from a yeast two-hydrid system in conjunction with a binding assay [26], with the NMR structure of the N-terminal domain of unbound CA [28], and with the crystal structure of the Fab–CA complex [29].

There are four proline residues in the 25-mer fragment (Pro85, Pro90, Pro93 and Pro99). While Pro85 and Pro99 were not visible, Pro90 and Pro93 were seen to have a *trans* peptide conformation. The *trans* peptide conformation of Pro90 in the crystal structure is comparable with the conformation of the unbound N-terminal domain of CA [28] and the 25-mer (U Reimer *et al.*, unpublished data) observed in NMR structures, but is in contrast to the *cis* conformation of proline-containing peptides in complex with CyPA [32,37,43]. On the other hand, Gly89 of the 25-mer had unusual backbone conformational angles of $\phi = 133^\circ$ and $\psi = 160^\circ$; this unfavorable backbone conformation can only be adopted by glycine.

The 12 amino acids of the 25-mer that could be traced comprised an extended backbone of Val86–Ala92 and a 3_{10} helix of Pro93–Arg97 (Table 2). The extended conformation of Val86–Ala92 is similar to the NMR solution structure of the N-terminal domain of unbound CA. However, Ala92–Gln95 adopted a type II β turn in the solution structure [28], but a type III β turn in our crystal structure (Table 2). Moreover, the 3_{10} helix of Pro93–Arg97 observed in the crystal structure was not reported in the solution structure [28]. These differences could either reflect a change in the conformation of the 25-mer from free solution to the bound state or represent a false difference resulting from the limited resolution of the methods.

The strand from Ala88–Ile91 of the 25-mer forms the main interactions with CyPA, including six hydrogen

Table 1

Interactions between the 25-mer and CyPA.

25-mer	CyPA	Distance (Å)
Hydrogen bonds		
His87:ND1	Asn71:O	2.85
His87:NE2	Wat76:O	2.82
Ala88:N	Gly72:O	2.89
Ala88:O	Gln63:NE2	3.18
Ala88:O	Wat50:O	2.64
Gly89:N	Asn102:O	2.82
Gly89:O	Wat56:O	2.96
Pro90:O	Arg55:NH1	2.70
Pro90:O	Arg55:NH2	2.77
Ile91:O	Trp121:NE1	3.09
Ile91:N	Wat41:O	3.21
Gly94:O	Wat59:O	3.03
Gln95:OE1	Arg55:NH1	2.88
Arg97:O	Wat59:O	2.96
Hydrophobic or polar interactions*		
Val86	Thr73	
His87	Asn71, Gly72, Thr73	
Ala88	Gln63, Gly72, Asn102, Gln111	
Gly89	Arg55, Gln63, Ala101, Asn102	
Pro90	Arg55, Phe60, Met61, Gln63, Phe113, Leu122, His126	
Ile91	Phe60, Trp121	
Ala92	Ile57, Phe60	
Pro93	Phe60, Trp121	
Nle96	Trp121	

*A distance of 3.2–4.0 Å is defined as a hydrophobic or polar interaction.

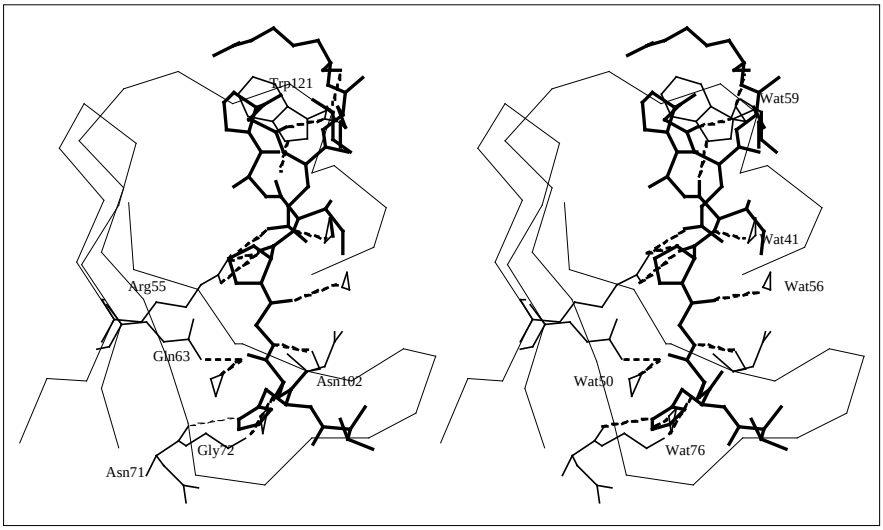
bonds and numerous hydrophobic contacts. A superposition between CyPA–25-mer and CyPA–AAPF, using only the CyPA structure as a probe, revealed that Ala88–Ile91 of the 25-mer corresponds to the tetrapeptide AAPF (Fig. 4). Pro90–Ile91 of the 25-mer and Pro3–Phe4 of AAPF occupy the same site, with small positional shifts of 0.18 and 0.28 Å for their Cα atoms and 0.45 Å for the proline sidechain. These residues also share the same pattern of hydrogen bonding. For example, their carbonyl oxygens both form hydrogen bonds with the sidechains of Arg55 and Trp121 (Table 1; [37]). However, Ala88–Gly89 of the 25-mer showed some dramatic differences from Ala1–Ala2 of AAPF: Ala88–Gly89 showed large Cα positional shifts of 2.4 and 2.7 Å (about ten times the average value of 0.27 Å for all Cα atoms of the structures); differences were seen in the interactions with CyPA; and the backbone conformation of the two peptides was not the same (Fig. 4; Table 2).

Is *cis-trans* isomerization involved in HIV-1 infectivity?

The unusual bound conformation of the 25-mer in the crystal structure suggests that the peptidyl–prolyl *cis-trans* isomerase activity of CyPA is unlikely to be involved in HIV-1 activity. Firstly, the residue preceding proline, Gly89, has a backbone conformation of $\phi = 133°$ and $\psi = 160°$, located in the energy-disallowed region of the Ramachandran plot [38]. This unfavorable backbone conformation is usually adopted only by glycine because of stereochemical restraints. Therefore, the binding of the HIV-1 gag protein to CyPA, which requires a special sequence of Gly–Pro, does not have the general character of *cis-trans* isomerization that presumably accepts a sequence with any types of amino acids preceding proline.

Secondly, the binding of Pro90, but not Pro85, Pro93 or Pro99 of the 25-mer, implies that the interactions between gag CA and CyPA are sequence specific. If *cis-trans* isomerization of a peptidyl–prolyl bond played a key role in HIV-1 gag protein folding, it might be predicted that the

Figure 3



Stereo plot of the active site of CyPA complexed with the 25-mer fragment. Thin lines represent the backbone traces of Ser52–Phe65 and Gly97–His126 and some sidechains of the active-site residues of CyPA; thick lines represent the residues of the gag 25-mer fragment. Water molecules (Wat) are marked as small triangles and dotted lines represent hydrogen bonds between the 25-mer, water molecules, and protein atoms. (The detailed interactions are listed in Table 1.)

Table 2

The backbone conformations of the 25-mer and AAPF (°).

25mer				AAPF			
Residue	ϕ	ψ	ω	Residue	ϕ	ψ	ω
Val86		–44	173				
His87	–95	132	177				
Ala88	–72	164	170	Ala1	–82	–38	180
Gly89	133	160	176	Ala2	–88	148	18
Pro90	–65	147	179	Pro3	–81	146	180
Ile91	–78	125	–179	Phe4	–93	129	–176
Ala92	–71	150	165				
Pro93	–42	–41	179				
Gly94	–71	–28	177				
Gln95	–78	–21	180				
Nle96	–89	–22	175				
Arg97	–144	–54					

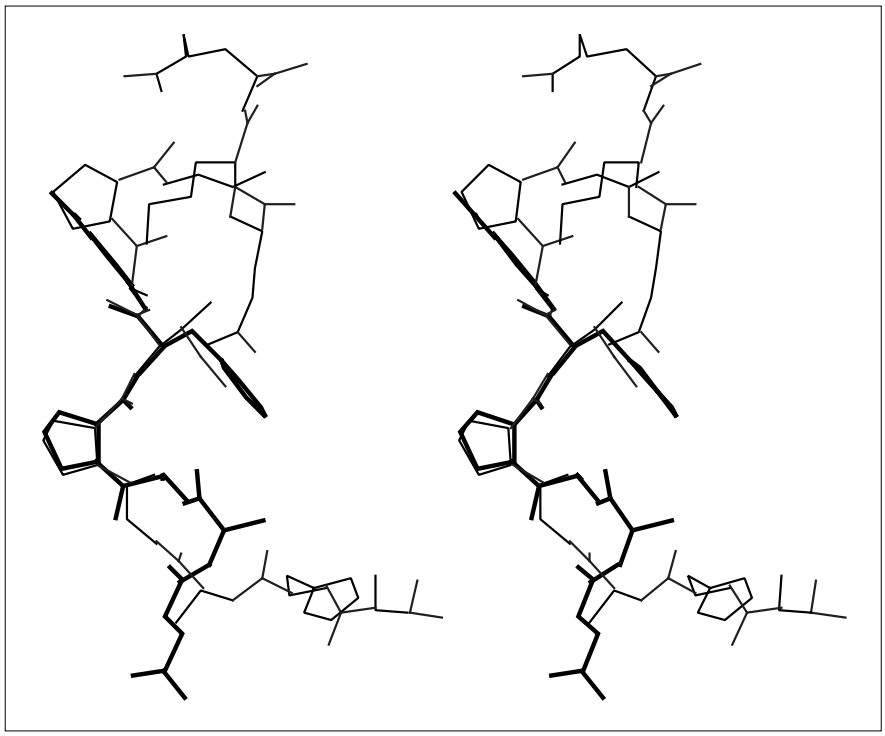
four prolines of the 25-mer would bind equally to the active site of CyPA. Guidance for this prediction is provided by the crystal structure, which reveals that interactions between the 25-mer and CyPA mainly involve the CyPA backbone atoms (Ala88–Ile91) and the sidechain of Pro90 of the 25-mer (Table 2).

Several lines of evidence from genetic and biochemical studies support the above argument. Site-directed mutagenesis showed that the mutation of Pro90 to alanine abolished the binding of full length gag to CyPA, whereas mutation of the other seven prolines to alanine had no effect on the binding [26]. Furthermore, mutation of Gly89 to alanine completely abolished gag binding to CyPA [26,27], suggesting that binding has an absolute requirement for glycine preceding proline. Finally, experiments which utilized mutagenesis, centrifugation, and electron microscopy revealed that CyPA is not required for HIV-1 virion assembly [27].

In summary, crystallographic and genetic evidence suggests that the binding of the gag protein to CyPA is a consequence of the special combination of glycine–proline. Therefore it is likely that in HIV-1 infectivity CyPA functions as a chaperone or an essential functional component, rather than acting as a *cis-trans* isomerase. However, the involvement of the *cis-trans* isomerization activity of CyPA in HIV-1 infectivity cannot be completely ruled out, because the crystal structure showed some similarities between the 25-mer and substrate AAPF. For example, both the 25-mer and the substrate AAPF have the same N to C polypeptide chain direction, the same hydrogen-bonding pattern of the C terminus, and a similar sidechain conformation of proline. If the C terminus of the 25-mer (residues after Pro90) plays a key role in the HIV-1 infectivity, CyPA might help the folding of the HIV-1 gag protein by *cis-trans* isomerization. Whether the Gly89–Pro90 bond in the 25-mer peptide can undergo catalysis of *cis-trans* isomerization by CyPA is under investigation.

Figure 4

Superposition of the 25-mer fragment (thin lines) and the peptide AAPF (thick lines). This figure has an orientation similar to Figure 3, with the N terminus of the peptides at the bottom and the C terminus at the top. The superposition matrix was obtained by superimposing the CyPA structures. The C-terminal portion of AAPF (Pro–Phe) obviously has the same binding conformation as the 25-mer while the N terminus of AAPF diverged because Gly89 of the 25-mer adopted an unusual backbone conformation ($\phi = 133°$ and $\psi = 160°$).



Biological implications

Cyclophilin A is a peptidyl–prolyl *cis-trans* isomerase and also binds the immunosuppressive drug cyclosporin A (CsA). The peptidyl–prolyl *cis-trans* isomerization activity of CyPA has been assumed to play a role in protein folding [8]. On the other hand, cyclophilins have been reported to function as chaperones binding several proteins including rhodopsin [17] and heat shock proteins [18]. CyPA is also known to be required for the infectious activity of human immunodeficiency virus type 1 (HIV-1) [20–22]. However, it is not understood whether the *cis-trans* isomerization activity of CyPA plays a role in the HIV-1 virion activity. The structure of CyPA complexed with a 25 amino acid fragment of the HIV-1 gag capsid protein may illustrate the role of CyPA in the HIV-1 activity.

The crystal structure of CyPA–25-mer revealed that Gly89 of the 25-mer has an unfavorable backbone conformation ($\phi = 133°$ and $\psi = 160°$); this conformation is usually only adopted by a glycine residue. The peptide bond between Gly89 and Pro90 has a *trans* conformation, in contrast to the *cis* conformation observed in other CyPA–peptide structures [32–37]. These observations suggest that the binding of CyPA to the gag protein requires a special sequence combination of glycine–proline, thus the *cis-trans* isomerase activity of CyPA is unlikely to be involved in the HIV-1 infectious activity. Genetic and biochemical studies showed that CyPA is required for HIV-1 replication and infectivity, but not for HIV-1 virion assembly [26,27]. Therefore, CyPA probably functions as a chaperone or a functional component, and not as a *cis-trans* isomerase, in HIV-1 infectivity. However, the involvement of the *cis-trans* isomerization activity of CyPA in the function of HIV-1 virions cannot be completely ruled out, because the 25-mer and the isomerase substrate succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide share some similarities in their C-terminal residues.

Materials and methods

The 25-mer of HIV-1 gag protein was synthesized as described elsewhere [44]. It has an amino acid sequence of Ac-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile-Ala-Pro-Gly-Gln-Nle-Arg-Glu-Pro-Arg-Gly-Ser-Asp-Ile-Ala-NH_2 corresponding to the sequence numbers 81–105 of the HIV gag CA except for Nle96 (norleucine) in the 25-mer but Met96 in the CA sequence [20].

Recombinant human CyPA was purified as previously described [45]. The complex of CyPA–25-mer was prepared by mixing the 25-mer of HIV-1 gag CA with CyPA in a molecular ratio of 1:1. Cocrystals of the CyPA–25mer complex were grown at room temperature by the vapor diffusion method. The well buffer was 90 mM Tris-HCl (pH 8.2), 41% saturated ammonium sulfate (AS), and 4.5 mM sodium azide. The protein drop contained 50 mM Tris-HCl (pH 8.2), 6% saturated AS, and 0.32 mM CyPA and the 25-mer. The crystals were formed in 2–4 days and diffracted extremely well. The space group was $P2_12_12_1$, with cell dimensions of $\alpha = 44.3$, $\beta = 53.1$, and $\gamma = 68.5$ Å. One molecule of the CyPA–25-mer complex exists in the crystallographic asymmetric unit, giving a Vm (volume of unit cell/molecular weight) of 1.92 $Å^3$ per dalton. The solvent content in the crystal is estimated to be in a range of 20–25%. Thus, the CyPA–25-mer complex is one of the most dense protein crystals.

Diffraction data were collected at room temperature on the Rigaku phosphate image plate system R-AXIS II and processed by the R-AXIS software. A total of 58 111 measurements were merged and reduced to 14 651 independent reflections with an R_{merge} of 0.0675. This data set is 94.3% complete to 1.8 Å resolution (Table 3).

The structure of the CyPA–25-mer complex was determined by the molecular replacement program AMoRe [46], using unligated CyPA as the initial model. The cross-rotation function of AMoRe resulted in two peaks. The translation search on the second peak of the cross-rotation function yielded a solution with a correlation coefficient of 0.589 and an R factor of 0.371 for data within the resolution range of 8–4 Å. The refinement on the rotation and translation by AMoRe yielded a correlation coefficient of 0.839 and an R factor of 0.340 for data between 15–2 Å resolution.

The solution from molecular replacement was first refined as a rigid body by the program X-PLOR [47] on a Dec Alpha server, yielding an R factor of 0.376 for the data at 8–1.8 Å resolution. Subsequently, the structure which included only CyPA was refined by positional and B factor refinement on individual atoms to an R factor of 0.281 (for data at 8–1.8 Å resolution). Two electron-density maps with coefficients of $(2F_o–F_c)$ and $(F_o–F_c)$ were calculated from this model and consistently revealed the binding and the Cα trace of the 25-mer. The model was built using the program FRODO/TOM [48] linked to a Silicon graphics system. After adding 12 amino acids of the 25-mer, further positional and B factor refinement brought the R factor to 0.243. Solvent molecules were then automatically picked up and incorporated into the

Table 3

Data collection and refinement statistics for the structure of CyPA–25-mer.

Data collection statistics	
Space group	$P2_12_12_1$
Cell a / b / c (Å)	44.3 / 53.1 / 68.5
Total measurements	58 111
Unique reflections	14 651
R_{merge} (Å)*	0.0675
Completeness (%)	
overall†	94.2
outer shell‡	88.3
Molecular replacement††	
α / β / γ (°)	83 / 50 / 243
Tx / Ty / Tz	0.238 / 0.013 / 0.148
Correlation coefficient	0.839
R factor§	0.34
Refinement statistics	
Resolution range (Å)	8.0–1.8
No. of residues CyPA	165 (1266 atoms)
No. of residues 25-mer	12 (86 atoms)
No. of waters	89
R factor	0.195
R_{free}#	0.256
Rms	
bond (Å)	0.014
angle (°)	2.8

*R_{merge} calculated for data at 1.8 Å resolution. †Completeness for data at 1.8 Å resolution; ‡completeness for the shell 2–18 Å. ††As defined in AmoRe [46]. §R_{factor} for data between 15–2 Å. #R_{free} is equivalent to the R factor for a randomly selected 8% subset of reflections not used in structure refinement.

refinement. After each round of refinement, water molecules with a high B factor were deleted, and new solvent molecules were picked up from the (F_o–F_c) map and added into the refinement. This water picking refining procedure was repeated until no peaks stronger than 3.5σ were found in the (F_o–F_c) map. The final structure includes 165 residues of CyPA, 12 residues of the gag 25-mer, and 89 water molecules; the structure has an R factor of 0.195 and R_{free} of 0.256 for 14 454 reflections between 8 to 1.8 Å resolution (Table 3). The structure comparison was performed with the program SUPERIMP or X-PLOR.

Accession numbers

The atomic coordinates have been deposited in the Protein Data Bank with accession code 1FGL.

Acknowledgements

We would like to thank Dr Christopher P Hill for his unpublished manuscript on the structure of CyPA complexed with the N-terminal domain of HIV-1 capsid. We also thank Mario Drewello for technical help with the synthesis of the 25-mer and Dr Richard Wolfenden for proof reading of the manuscript. This work was partially supported by NIH grant AI33072 to HK.

References

1. Handschumacher, R.E., Harding, M.W., Rice, J. & Drugge, R.J. (1984). Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science* **226**, 544–547.
2. Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T. & Schmid, F.X. (1989). Cyclophilin and peptidyl-prolyl *cis-trans* isomerase are probably identical proteins. *Nature* **337**, 476–478.
3. Takahashi, N., Hayano, T. & Suzuki, M. (1989). Peptidyl–prolyl *cis-trans* isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* **337**, 473–475.
4. Klee, C.B., Draetta, G.F. & Hubbard, M.J. (1988). Calcineurin. *Adv. Protein Chem.* **61**, 149–199.
5. Liu, J., Farmer, J.D., Jr., Lane, W.S., Friedman, J., Weissman, I. & Schreiber, S.L. (1991). Calcineurin is a common target of cyclophilin–cyclosporin A and FKBP–FK506 complexes. *Cell* **66**, 807–815.
6. Friedman, J. & Weissman, I. (1991). Two cytoplasmic candidates for immunophilin action are revealed by affinity for a new cyclophilin: one in the presence and one in the absence of CsA. *Cell* **66**, 799–806.
7. Fischer, G. & Schmid, F.X. (1990). The mechanism of protein folding. Implications of *in vitro* refolding model for *de novo* protein folding and translocation in the cell. *Biochemistry* **29**, 2205–2212.
8. Schmid, F.X., Mayr, L.M., Mucks, M. & Schönsbrunner, E.R. (1993). Prolyl isomerization: role in protein folding. *Adv. Protein Chem.* **44**, 25–66.
9. Bächinger, H.P. (1987). The influence of peptidyl–prolyl *cis-trans* isomerase on the *in vitro* folding of type III collagen. *J. Biol. Chem.* **262**, 17144–17148.
10. Davis, J.M., Boswell, B.A. & Bächinger, H.P. (1989). Thermal stability and folding of type IV procollagen and effect of peptidyl–prolyl *cis-trans* isomerase on the folding of the triple helix. *J. Biol. Chem.* **264**, 8956–8962.
11. Fransson, C., *et al.*, & Carlsson, U. (1992). *Cis-trans* isomerization is rate-determining in the reactivation of denatured human carbonic anhydrase II as evidenced by proline isomerase. *FEBS Lett.* **296**, 90–94.
12. Jackson, S.E. & Fersht, A.R. (1991). Folding of chymotrypsin inhibitor 2. 2. Influence of proline isomerization on the folding kinetics and thermodynamic characterization of the transition state of folding. *Biochemistry* **30**, 10436–10443.
13. Kiefhaber, T., Quaas, R., Hahn, U. & Schmid, F.X. (1990). Folding of ribonuclease T1. 1. Existence of multiple unfolded states created by proline isomerization. *Biochemistry* **29**, 3053–3061.
14. Lang, K., Schmid, F.X. & Fischer, G. (1987). Catalysis of protein folding by prolyl isomerase. *Nature* **329**, 268–270.
15. Schönbrunner, E.R., Mayer, S., Tropschug, M., Fischer, G., Takahashi, N. & Schmid, F.X. (1991). Catalysis of protein folding by cyclophilins from different species. *J. Biol. Chem.* **266**, 3630–3635.
16. Steinmann, B., Bruchner, P. & Superti-Furga, A. (1991). Cyclosporin A slows collagen triple-helix formation *in vitro*: indirect evidence for a physiologic role of peptidyl–prolyl *cis-trans* isomerase. *J. Biol. Chem.* **266**, 1299–1303.
17. Baker, E.K., Colley, N.J. & Zuker, C.S. (1994). The cyclophilin homolog NinaA functions as chaperone, forming a stable complex *in vivo* with its protein target rhodopsin. *EMBO J.* **13**, 4886–4895.
18. Johnson, J.L. & Toft, D.O. (1994). A novel chaperone complex for steroid receptors involving heat shock proteins, immunophilins, and p23. *J. Biol. Chem.* **269**, 24989–24993.
19. Luban, J., Bossolt, K.L., Franke, E.K., Kalpana, G.V. & Goff, S.P. (1993). Human immunodeficiency virus type 1 gag protein binds to cyclophilins A and B. *Cell* **73**, 1067–1078.
20. Franke, E.K., Yuan, H.E.H. & Luban, J. (1994). Specific incorporation of cyclophilin A into HIV-1 virions. *Nature* **372**, 359–362.
21. Thall, M., *et al.*, & Göttlinger, H.G. (1994). Functional association of cyclophilin A with HIV-1 virions. *Nature* **372**, 363–365.
22. Braaten, D., Franke, E.K. & Luban, J. (1996). Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and simiam immunodeficiency virus SIVcpzGAB but not group O HIV-1 or other primate immunodeficiency viruses. *J. Virol.* **70**, 4220–4227.
23. Wills, J.W. & Craven, R.C. (1991). Form, function, and use of retroviral gag proteins. *AIDS* **5**, 639–654.
24. Gelderblom, H.R. (1991). Assembly and morphology of HIV: potential effect of structure on viral function. *AIDS* **5**, 617–638.
25. Franke, E.K., Chen, A., Tatsis, I., Diamanduros, A., Erlanger, B. & Luban, J. (1995). Cyclophilin binding to human immunodeficiency virus type 1 gag polyprotein is mimicked by an anti-cyclosporin antibody. *J. Virol.* **69**, 5821–5823.
26. Colgan, J., Yuan, H.E.H., Franke, E.K. & Luban, J. (1996). Binding of human immunodeficiency virus type 1 gag polyprotein to cyclophilin A is mediated by the central region of capsid and requires gag dimerization. *J. Virol.* **70**, 4220–4227.
27. Braaten, D, Franke, E.K. & Luban, J. (1996). Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. *J. Virol.* **70**, 3551–3560.
28. Gitti, R.K., Lee, B.M., Walker, J., Summers, M.F., Yoo, S. & Sundquis, W.I. (1996). Structure of amino-terminal core domain of the HIV-1 capsid protein. *Science* **273**, 231–235.
29. Momany, C., *et al.*, & Rossmann, M.G. (1996). Crystal structure of dimeric HIV-1 capsid protein. *Nat. Struct. Biol.* **3**, 763–770.
30. Ke, H., Zydowsky, L.D., Liu, J. & Walsh, C.T. (1991). Crystal structure of recombinant human T-cell cyclophilin A at 2.5 Å resolution. *Proc. Natl. Acad. Sci. USA* **88**, 9483–9487.
31. Ke, H. (1992). Similarities and differences between human cyclophilin A and other β-barrel structures: structural refinement at 1.63 Å resolution. *J. Mol. Biol.* **228**, 539–550.
32. Kallen, J., *et al.*, & Walkinshaw, M.D. (1991). Structure of human cyclophilin and its binding site for cyclosporin A determined by X-ray crystallography and NMR spectroscopy. *Nature* **353**, 276–279.
33. Kallen, J. & Walkinshaw, M.D. (1992). The X-ray structure of a tetrapeptide bound to the active site of human cyclophilin A. *FEBS Lett.* **300**, 286–290.
34. Ke, H.M., Mayrose, D. & Cao, W. (1993). Crystal structure of cyclophilin A complexed with substrate Ala–Pro suggests a solvent-assisted mechanism of *cis-trans* isomerization. *Proc. Natl. Acad. Sci. USA* **90**, 3324–3328.
35. Zhao, Y. & Ke, H.M. (1996). Mechanistic implication of crystal structures of the cyclophilin-dipeptide complexes. *Biochemistry* **35**, 7362–7368.
36. Konno, M., Ito, M., Hayano, T. & Takahashi, N. (1996). The substrate-binding site in *Escherichia coli* cyclophilin A preferably recognizes a *cis*-proline isomer or a highly distorted form of the *trans* isomer. *J. Mol. Biol.* **256**, 897–908.
37. Zhao, Y. & Ke, H.M. (1996). Crystal structure implies that cyclophilin predominantly catalyzes the *trans* to *cis* isomerization. *Biochemistry* **35**, 7356–7361.
38. Ramachandran, G.N. & Sasisekharan, V. (1968). Conformation of polypeptide and proteins. *Adv. Protein Chem.* **23**, 283–437.
39. Ke, H., *et al.*, & Walsh, C.T. (1994). Crystal structures of cyclophilin A complexed with cyclosporin A and *N*-methyl-4-[(E)-2-butenyl]-4,4-dimethyl-threonine cyclosporin A. *Structure* **2**, 33–44.
40. Thèriault, Y., *et al.*, & Fesik, S.W. (1993). Solution structure of the cyclosporin A/cyclophilin complex by NMR. *Nature* **361**, 88–91.
41. Pflügl, G., Kallen, J., Schirmer, T., Jansonius, J.N., Zurini, M.G.M. & Walkinshaw, M.D. (1993). X-ray structure of a decameric cyclophilin–cyclosporin crystal complex. *Nature* **361**, 91–94.
42. Mikol, V., Kallen, J., Pflügl, G. & Walkinshaw, M.D. (1993). X-ray structure of a monomeric cyclophilin A–cyclosporin A crystal complex at 2.1 Å resolution. *J. Mol. Biol.* **234**, 1119–1130.
43. Kakalis, L.T. & Armitage, I.M. (1994). Solution conformation of a cyclophilin-bound proline isomerase substrate. *Biochemistry* **33**, 1495–1501.

44. Schutkowski, M., *et al*., & Fischer, G. (1996). Extended binding sites of cyclophilin as revealed by the interaction with HIV-1 gag polyprotein derived oligopeptide. *FEBS Lett.* 394, 289–294.
45. Liu, J., Albers, M.W., Chen, C.M., Schreiber, S.L. & Walsh, C.T. (1990). Cloning, expression, and purification of human cyclophilin in *Escherichia coli* and assessment of the catalytic role of cysteines by site-directed mutagenesis. *Proc. Natl. Acad. Sci. USA* 87, 2304–2308.
46. Navaza, J. (1994). AMoRe, an automated package for molecule replacement. *Acta Cryst. A* 50, 157–163.
47. Brünger, A.T., Kuriyan, J. & Karplus, M. (1987). Crystallographic R factor refinement by molecular dynamics. *Science* 235, 458–460.
48. Jones, T.A. (1982). In *Computational Crystallography*. (Sayer, D., ed.), pp. 303–317, Oxford University Press, UK.
49. Nicholls, A., Bharadwaj, R. & Honig, B. (1993). GRASP: graphic representation and analysis of surface properties. *Biophys. J.* 64, 166–170.
50. Kraulis, P.J. (1991). MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Cryst.* 24, 946–950.
51. Merritt, E.A. & Murphy, M. (1994). Raster3D version 2.0 – a program for photorealistic molecular graphics. *Acta Cryst. D* 50, 869–873.